BIOCONVERSION OF METHANE TO METHANOL BY METHYLOBACTERIUM ORGANOPHILUM.

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INTRODUCTION

Large reserves of natural gas have stimulated the development of processes that can convert methane to more valuable chemicals such as methanol. Commercial routes to methanol involve three steps. First synthesis gas is generated from natural gas or naphtha at 15-30 atmospheres and 840-900 °C. The synthesis gas is converted to methanol using a copper catalyst which requires the process condition of 300-350 psig and 250-270 °C. The methanol is then distilled to desired purity. Many bacteria and fungi grow on methane at ambient temperature and pressure. In this study we attempt to develop a low severity route from methane to methanol, involving a biochemical catalyst.

Methylobacterium organophilum was grown in a methane-oxygen controlled atmosphere water bath shaker apparatus to study the bioconversion of methane to more valuable chemicals such as methanol. To optimize production of methanol from the metabolism of methane by Methylobacterium organophilum, we tested the effects of culture enrichment and inhibitors.

EXPERIMENTAL

Methylobacterium organophilum was purchased from the American Type Culture Collection (ATCC #27886). For all studies, excepted when noted, ammonium mineral salts (AMS) medium was used.

All growth experiments were conducted under a methane atmosphere, in a controlled atmosphere water bath shaker apparatus. Liquid cultures were grown in 250 ml Erlenmeyer flasks at 30 °C on the rotary shaker (150-200 rpm) at pH 6.8 with methane as the only carbon source for growth (unless otherwise stated). The atmosphere of the incubator shaker was normally continuously gassed with 65% methane, 20% oxygen, and 15% nitrogen.

Cell densities were measured by monitoring the absorbance at 660nm by Sargent-Welch model SM spectrophotometer. The cells were harvested by centrifugation and dry cell weights were determined after drying the cell paste in a vacuum oven.

Cell free culture broth was analyzed for methanol by gas chromatography. The cells were removed by centrifugation followed by filtration.

RESULTS AND DISCUSSION

Methylobacterium organophilum is a facultative methylotroph; it can grow not only on C-1 compounds but also on multicarbon compounds as the sole sources of carbon and energy. Figure 1 shows the growth of M. organophilum on one-carbon and multicarbon substrates.

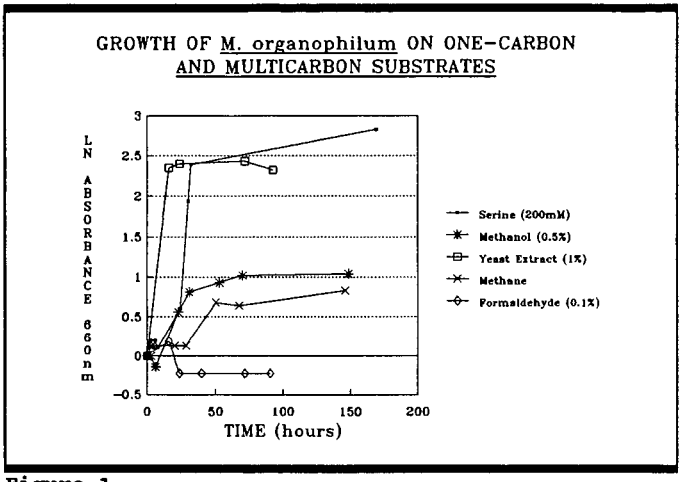

Figure 1

The specific growth rate on methane is lower than the other substrates. This is expected since the solubilities in water of the other substrates are higher than the solubility of methane in water.

Another important characteristic of M. organophilum is the type II intracytoplasmic membrane. Cultures previously grown on a multicarbon substrate for growth and energy required several transfers grown on methane before accumulating methanol. This "inactive" state of the microbe may be explained by the fact that it is necessary for the bacterium to possess an intracytoplasmic membrane for methane metabolism to occur. The literature reports that M. organophilum contains an intracytoplasmic membrane when grown on methane but this membrane is not present during growth on higher substrates such as methanol and glucose (1).

Methylobacterium organophilum oxidizes methane by a special C-1 oxidation pathway (see below).

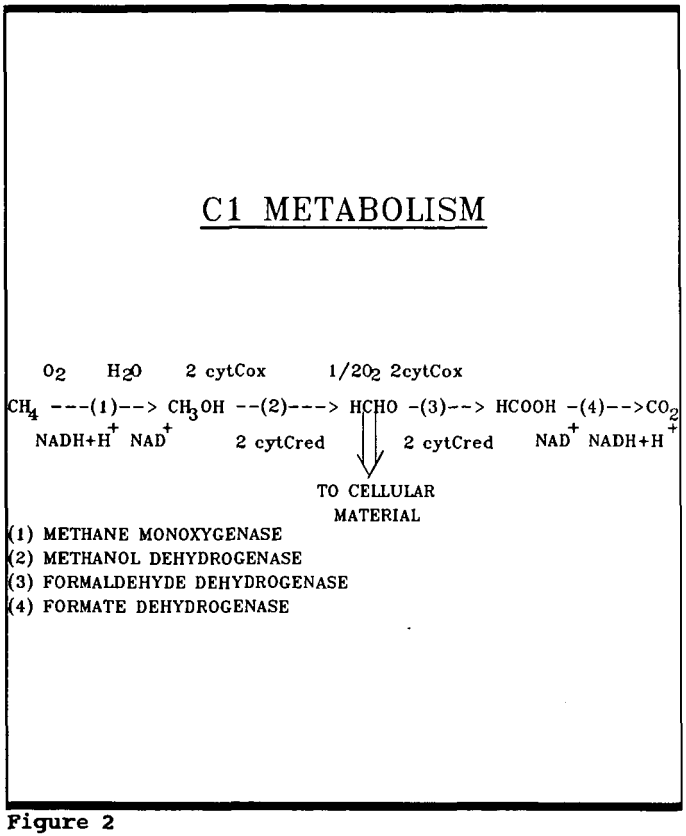

Figure 2

Biomass is produced through formaldehyde assimilation. The mechanism of the reaction CH_4 -----> CH_3OH involves atmospheric oxygen incorporated directly into the methane molecule with the aid the enzyme, monooxygenase. The hydrogen requirement is supplied by the conversion of formic acid to carbon dioxide implying that methane oxidation is a function of successive oxidations. This presents a problem if methane oxidation is stopped at methanol because the regeneration of NAD+ would be lost.

High biomass cultures of M. organophilum were tested for methanol accumulation while growth was monitored (see below).

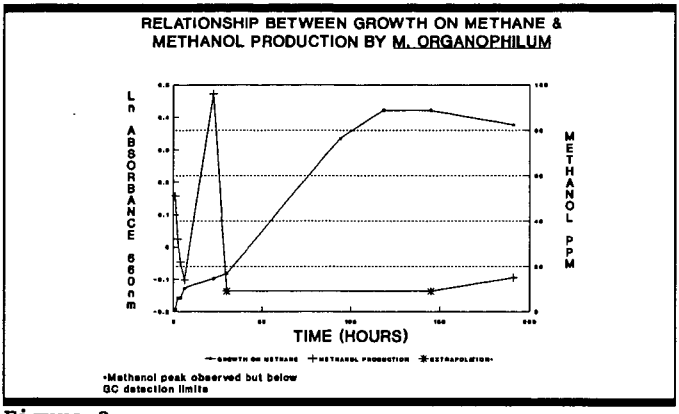

Figure 3

It appears that methanol accumulates during non-growth periods from methane oxidation. This is expected since during growth, the methanol is further metabolized for cell growth and energy. During initial incubation, methanol production was low (1.1 mmoles/gDCW.Hr).

The effect of iodoacetic acid on methanol production was studied. Cultures of M. organophilum were tested for methanol accumulation during growth on methane in the presence of iodoacetic acid (see below).

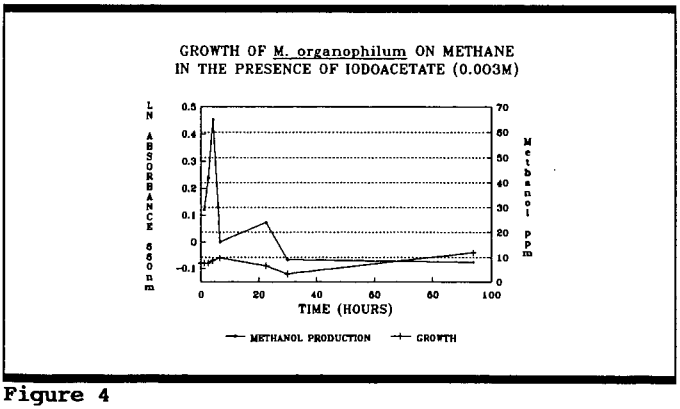

Figure 4

Iodoacetic acid inhibited growth but did not inhibit further oxidation of methanol. Iodoacetic acid had no effect on methanol accumulation at the described conditions.

Culture enrichment was also studied to increase the methanol production rate. M. organophilum was grown on serine in the presence of methane. Serine is an intermediate metabolite in the pathway of methane oxidation to cellular material. By adding high concentrations of serine (200mM) in a culture of M. organophilum growing on methane, the equilibrium of the organism may shift. Creation of a metabolic block through manipulation of the environment (the addition of serine) may bring accumulation of metabolites preceeding the block. Methane (the original carbon source) is the starting material for bioconversion (Figure 5).

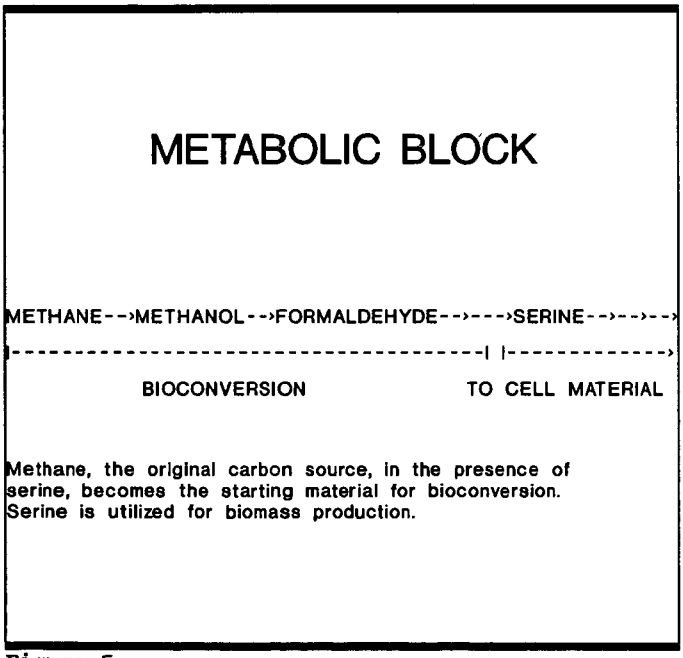

Figure 5

During growth on methane and serine, accumulation of formic acid and acetic acid were seen during growth. Volumetric rates were 0.1g/l.hr and 0.3g/l.hr for formic acid and acetic acid, respectively.

Figure 6 shows that regulation in M. organophilum is obvious during growth on more than one carbon substrate in the medium.

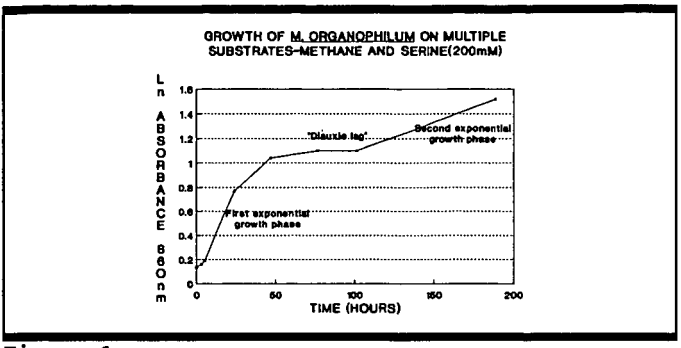

Figure 6

Growth of M. organophilum on methane and serine shows the diauxie phenomenon discovered by Monad(2). The substrates are utilized in two exponential growth cycles. The growth cycles are separated by an intermediate lag phase.

Formic acid could be a product produced by a metabolic block created by the addition of serine since the following part of the pathway precedes serine.

Methane---> Methanol---> Formaldehyde---> Formic Acid

However, formic and acetic acid are also produced by M. organophilum during serine fermentation void of methane (see below).

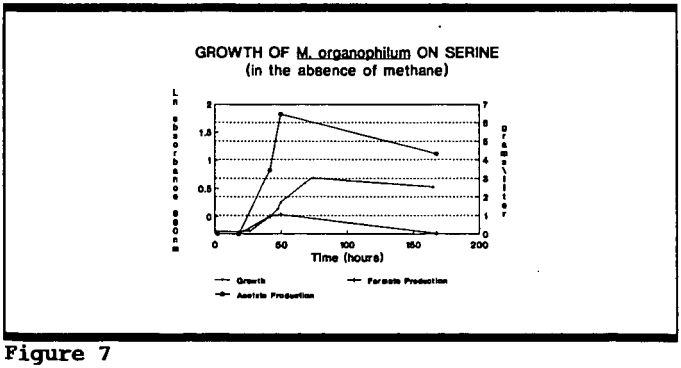

Figure 7

Waber et al (3) have shown the conversion of serine to pyruvate, which is decarboxylated to form acetate by Clostridium acidiurici. Kobota (4) found that serine generated formic acid form a cell-free preparation form Bacillus brevis in the presence of a cofactor, tetrahydrofolic acid. Tetrahydrofolate is present in M. organophilum and is used for 1-carbon group transfer and reduction.

CONCLUSIONS

Methanol accumulated during non-growth periods of methane metabolism by M. organophilum in the presences and absence of inhibitors under the conditions described. The production rate was increased by varying experimental conditions. For example, methanol accumulation increased by a factor of ca. 4 by pregrowing the culture in the presence of an inhibitor. When compared to commercial processes of methanol production, the bioconversion of methanol is ca. 90 times less active. Increasing methanol yields by optimizing culture medium and growth condition is limited by the natural isolate of Methylobacterium organophilum ability to synthesize methanol. Successful exploitation of Methylobacterium organophilum requires the application of genetic techniques for the optimization of methanol production.

Formic acid and acetic acid were growth associated products of serine fermentation by Methylobacterium organophilum.

REFERENCES

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